

Research Article

# A Study on the Effect of Integrated Ozone and UVC-LED Approaches on the Reduction of *Salmonella typhimurium* Bacteria in Droplets

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**ABSTRACT** In the wake of the SARS-CoV-2 pandemic, inactivating bioaerosols became a pivotal issue which helps to prevent the transmittance of SARS-CoV-2. Thus, the current study was conducted to investigate a potential inactivating method using both ozone (O<sub>3</sub>) and ultraviolet C (UVC). Individual and integrated effects of O<sub>3</sub> and UVC were compared. A solution containing approximately  $4\sim 7.3 \times 10^6$  CFU/mL of *Salmonella typhimurium* bacteria was used to produce bacteria droplets. These droplets were exposed to O<sub>3</sub> and UVC to determine the reduction rate of bacteria. The exposure times were set as 1 and 30 minutes. Ozone concentrations were 100 and 200 ppmv. UVC-LEDs were used as a UVC source. Peak wavelength of the UVC-LED was 275 nm and the irradiation dose was 0.77 mW/cm<sup>2</sup>. In terms of O<sub>3</sub> and UVC-LED interaction, 194 ppmv styrene was used as a target compound to be removed. Considering the O<sub>3</sub> and UVC-LED interaction, the presence of O<sub>3</sub> could reduce the performance of the UVC-LED, and UVC-LED could also reduce significant amount of O<sub>3</sub>. The sequence of O<sub>3</sub> and UVC-LED treatment was as follows: O<sub>3</sub> was exposed at first, then UVC-LED, and this order showed the best reduction ratio (> 99.9%). Therefore, if O<sub>3</sub> and UVC-LED is used to disinfect *Salmonella typhimurium* bacteria contained in droplets, bacteria should be separately exposed to O<sub>3</sub> and UVC-LED in order to improve the inactivation efficiency.

**KEY WORDS** UVC-LED, Ozone, *Salmonella typhimurium*, Disinfection, Styrene, Bioaerosol

## 1. INTRODUCTION

A COVID-19 pandemic has been occurring since 2020 and has not been ended (WHO, 2021a; Acuti Martellucci *et al.*, 2020). A syndrome coronavirus 2 (SARS-CoV-2) is the core reason of the pandemic (Qian *et al.*, 2021; US EPA, 2021; WHO, 2021a; Acuti Martellucci *et al.*, 2020; Morawska *et al.*, 2020). It was reported that the SARS-CoV-2 can be transmitted through aerosol which are respiratory droplets emitted through human exhalation such as coughing, sneezing, speaking, etc. Regarding to SARS-CoV-2 patents, their aerosols contain virus particles (Qian *et al.*, 2021; US EPA, 2021; WHO, 2021b; Acuti Martellucci *et al.*, 2020; Morawska *et al.*, 2020) so called bioaerosols (Stetzenbach, 2009). Moreover, since most people spend about 90% of their time in the indoor environment (da Costa Filho and Vilar, 2020; Kruza *et al.*, 2020), most of the SARS-CoV-2 patients were infected in the

indoor air, which was responsible for approximately 80% out of total infection cases (Qian *et al.*, 2021; Morawska *et al.*, 2020). Therefore, the improvement of indoor air quality is a pivotal issue (WHO, 2021c; Morawska *et al.*, 2020). A ventilation method and an air conditioning method are needed to reduce the infection rate (WHO, 2021c; Morawska *et al.*, 2020). The main function of these methods is to reduce the amount of bioaerosols from the indoor air or to inactivate bacteria and viruses in bioaerosols (Buising *et al.*, 2021; WHO, 2021c; Morawska *et al.*, 2020; Ren *et al.*, 2020). Ultraviolet C (UVC) (Ploydaeng *et al.*, 2021; Yoo *et al.*, 2021; Nunayon *et al.*, 2020; Ren *et al.*, 2020; Beck *et al.*, 2017; Wengraitis *et al.*, 2013) and ozone (O<sub>3</sub>) (Franke *et al.*, 2021; Sallustio *et al.*, 2021; Shi *et al.*, 2021; Steinmann *et al.*, 2021; Wang *et al.*, 2021; Masotti *et al.*, 2019; Huang *et al.*, 2012) have been widely applied to inactivate bacteria and viruses. However, the disinfection of bacteria and viruses in bioaerosols in the indoor air using UVC light-emitting diode (UVC-LED) has not been documented well (Nunayon *et al.*, 2020; Ren *et al.*, 2020).

In terms of bacteria, a rotating UVC-LED system consisting of 10 UVC-LEDs (271 nm) was installed at an upper position of a room (2.01 m of height) to inactivate bacteria in bioaerosols (Nunayon *et al.*, 2020). The irradiation of UVC-LEDs varied from 0.316 μW/cm<sup>2</sup> to 0.517 μW/cm<sup>2</sup>. The room dimension was 2.25 m × 2.30 m × 2.30 m. Colonies of *E. coli*, *S. marcescens*, and *S. epidermidis* bacteria were used to produce bioaerosols. It was found that *S. epidermidis* bacteria showed the highest UVC susceptible, followed by *S. marcescens* and *E. coli* bacteria. It was reported that it took 117~176 minutes in removing 99.99% of air-borne bacteria when the UVC-LEDs system was not rotated and that it took 77~91 minutes when the system was rotated (Nunayon *et al.*, 2020). In terms of O<sub>3</sub>, the oxidation property of O<sub>3</sub> is generally due to O\* radical when O<sub>3</sub> decomposes in the environment as the following equation (Batakiev *et al.*, 2014):



O\* is a strong oxidant which can quickly oxidize organic components of germs (Mohamed and Barbara, 2021). Several studies on inactivating bioaerosols in the indoor air were reported (Steinmann *et al.*, 2021; Masotti *et al.*, 2019; Huang *et al.*, 2012). Bioaerosols consisting of *E. coli* and *B. subtilis* bacteria were exposed to various concentrations of O<sub>3</sub> (i.e., 20 to 175 ppm) to

determine the bacteria reduction efficiencies (Huang *et al.*, 2012). The experiment was conducted with an environmental chamber and a control room. In terms of the environmental chamber, it was reported that the reduction efficiency of *E. coli* at 50 ppmv O<sub>3</sub> was 95% with 10 s of exposure time. In contrast, that of *B. subtilis* was approximately 0% at all levels O<sub>3</sub> of concern after 10 s contact. For the control room, the total bacteria including *E. coli* and *B. subtilis* was reduced from about 160 CFU/m<sup>3</sup> to about 10 CFU/m<sup>3</sup> (i.e., 93.7% of reduction rate) after 2h with an air change rate of 3.89 L/h and 150 ppmv O<sub>3</sub> (Huang *et al.*, 2012). O<sub>3</sub> with an average concentration of 5 ppmv was used to disinfect air-borne bacteria in a food factory (Masotti *et al.*, 2019). O<sub>3</sub> exposure time was three hours. The air in the testing room was in a stationary state. It was reported that the removal efficiency of bacteria with the abundance of *C. herbarum* was approximately 100% (Masotti *et al.*, 2019).

For viruses, Ren *et al.* (2020) claimed that a UVC-LED at 254 nm could disinfect SARS-CoV-2 in circulating air at a medical center (Ren *et al.*, 2020). However, more detailed information about the study was not addressed. Effects of 80 ppmv O<sub>3</sub> on adenovirus type-5 and murine norovirus were investigated. A testing room had a volume of 62.48 m<sup>3</sup> and its air was controlled with a relative humidity of 90%. Exposure times were 150 and 300 minutes. It was found that the reduction efficiency of adenovirus type-5 was over 99% after 150 minutes and over 99.9% after 300 minutes. In contrast, that of murine norovirus was only over 90% after 150 minutes and over 99% after 300 minutes (Steinmann *et al.*, 2021).

In general, UVC-LEDs or O<sub>3</sub> has a good potential to disinfect bioaerosols. They can be used only in the absence of humans because they are hazardous objects and their exposure times are long enough to achieve a high reduction efficiency (>99.9%). Thus, ventilation was the best method to reduce the infection of bioaerosols (Morawska *et al.*, 2020). A circulation rate was recommended from 2 to 50 air change per hour (Morawska *et al.*, 2020). However, the circulation air may transmit bioaerosols from indoor to outdoor environment and vice versa. Consequently, germs in bioaerosols in the circulation air should be inactivated in the ventilation system. Since the recommended ventilation rate is high, a strong and fast disinfection method should be used. An integrated O<sub>3</sub> and UVC-LED technique may be a potential method. However, there has been a lack of study on this issue.

Accordingly, this study was conducted to investigate the individual and integrated effects of O<sub>3</sub> and UVC-LED on the reduction of bacteria in liquid droplets. There are several methods to integrate O<sub>3</sub> and UVC-LED. Thus, a pre-experiment was carried out to determine the effect of various integrations between O<sub>3</sub> and UVC-LED on a reductant. Styrene was used as a representative reductant. Then, an optimal integrated method was applied to investigate its effect on bacteria in liquid droplets. *Salmonella typhimurium* was used as a representative bacterium. Reduction efficiencies of bacteria in droplets were determined with respect to various exposure times and the sequence of O<sub>3</sub> and UVC-LED treatment.

## 2. MATERIALS AND METHODS

### 2.1 Materials

Ozone was generated by an ozone generator (FOZ-5A, Fine Ozone, Republic of Korea). Oxygen (10.02%, Rigas, Republic of Korea) was used as an oxygen source of the ozone generator. Styrene (399 ppmv, Rigas, Republic of Korea) was used as a representative reductant for investigating interactions between UVC-LED and O<sub>3</sub>. Zero air (99.99%, DongA Ltd., Co., Republic of Korea) was used to dilute the styrene standard gas. *Salmonella typhimurium* bacterium solution containing  $4\sim 7.3 \times 10^6$  CFU/mL was used to investigate the effects of UVC-LED and O<sub>3</sub>.

### 2.2 Apparatus

An ozone generator (FOZ-5A, Fine Ozone, Republic of Korea) was used to produce ozone standard gas. An ozone analyzer (ANA4, Winstech Inc., Republic of Korea) was applied for the analysis of ozone. In terms of styrene analysis, a gas chromatography (GC) (6890, Agilent technologies, USA)/ Mass spectrometer (MS) (5975, Agilent technologies, USA) coupled with a thermal desorber (TD) (Unity 2, Markes international, UK). A capillary column (60 m × 0.320 mm × 1.80 μm) (DB-624, Agilent technologies, USA) was applied for the GC. The operation conditions of the GC/MS/TD system can be found elsewhere (Lee *et al.*, 2019a, 2019b). A Tenax TA trap (C1-AXXX-5003, Markes, UK) was used for the sampling of styrene. Ten UVC-LED modules (WOB\_16A, Seoul Viosys Co., Republic of Korea) with a peak wavelength at 275 nm were

installed in an isolated chamber where 5 modules were attached on the top and the rest were located on the bottom of the chamber. The distance between top and bottom UVC-LED modules was 6 cm because the manufacturer recommended 3 cm of effective distance. An UVC light meter (UVC-254SD, Lutron Electric Enterprise Co., Ltd., Taiwan) was used to measure the UVC intensity.

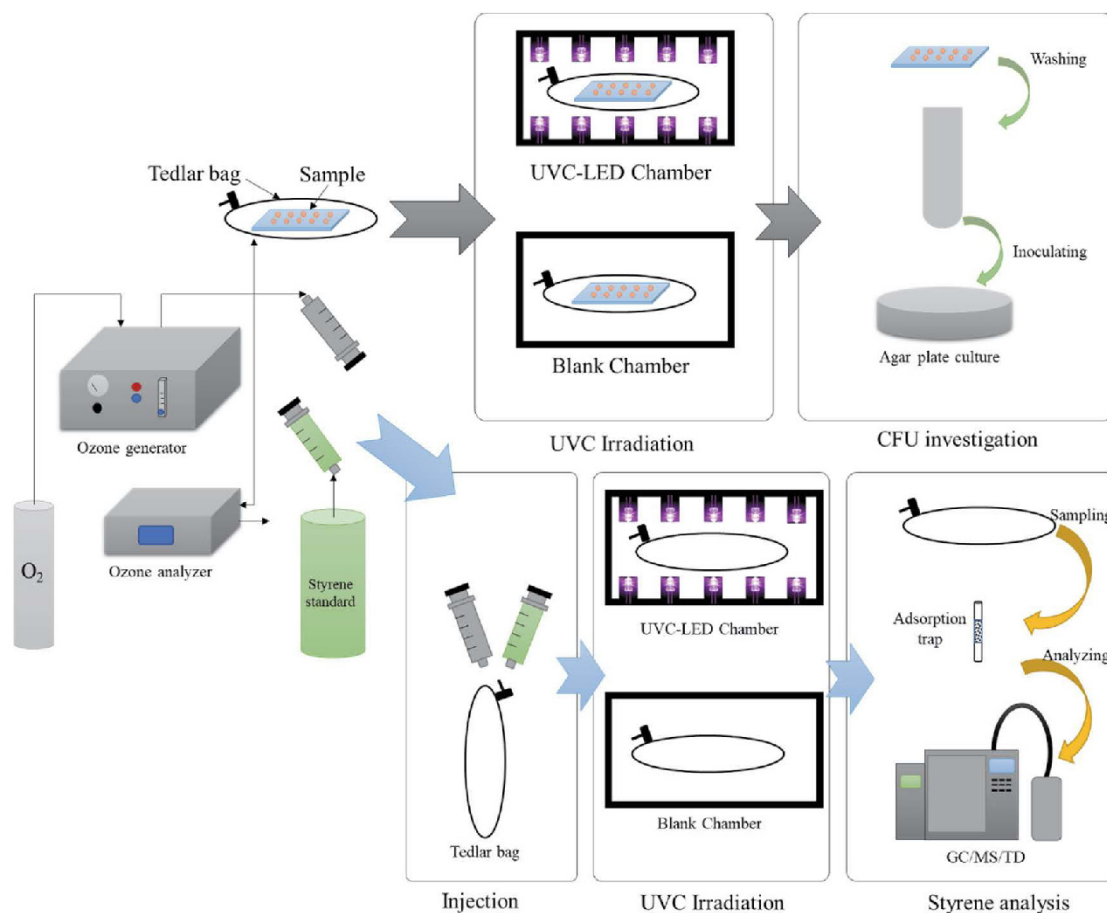
## 2.3 Experimental Procedures

### 2.3.1 Investigation of Interactions between O<sub>3</sub> and UVC-LED

In general, O<sub>3</sub> and UVC individually show a strong oxidizing property. However, an interaction between O<sub>3</sub> and UVC also occurs (Keller-Rudek *et al.*, 2013; Summerfelt, 2003; de Gruijil and van der Leun, 2000). It was reported that O<sub>3</sub> can strongly absorb UV in the range of 200 to 300 nm (Keller-Rudek *et al.*, 2013). Furthermore, UV in the range of 240 to 310 nm can decompose O<sub>3</sub> to be oxygen gas and oxygen radical (Summerfelt, 2003; de Gruijil and van der Leun, 2000). For the combination effects of O<sub>3</sub> and UVC-LED, an object can be exposed to O<sub>3</sub> and UVC-LED in various ways such as exposed by simultaneously by O<sub>3</sub> and UVC-LED, exposed by O<sub>3</sub>, then by UVC-LED, or exposed by UVC-LED, then by O<sub>3</sub>. Therefore, an optimal integrated method which shows practical performance should be determined.

First, the reduction rate of O<sub>3</sub> caused by UVC-LED was investigated. 500 mL of O<sub>3</sub> at 100 ppmv and 200 ppmv was respectively injected into 1 L Tedlar bags (SKC Inc., USA). Then, each bag was irradiated with UVC-LED at various exposure times including 1, 5, 10, 15 and 30 minutes. The reduction rates of O<sub>3</sub> were evaluated based on its concentrations before and after the irradiation. O<sub>3</sub> in ppmv levels (i.e., 5 ppmv to 175 ppmv) was studied, and it was reported that the chemical could disinfect bacteria in bioaerosols with respect to various exposure times from few seconds to few hours (Steinmann *et al.*, 2021; Masotti *et al.*, 2019; Huang *et al.*, 2012). Particularly, although 175 ppmv of O<sub>3</sub> was applied, it did not reveal a good reduction efficiency on *B. subtilis* in a short exposure time (Huang *et al.*, 2012). Therefore, O<sub>3</sub> levels were selected as 100 ppmv and 200 ppmv in this study.

Second, the optimal integration of O<sub>3</sub> and UVC-LED was investigated. For bacteria, since it generally takes a long time to inoculate, culture, and count them, a reduc-



**Fig. 1.** Experimental procedure for investigating effects of the integrated  $O_3$ /UVC-LED on the oxidation of styrene and droplets containing bacteria.

tant was used instead of a bacterium in this experiment to reduce the experimental time. In general, UVC can damage the double-bond stability between adjacent carbons and conjugated ring structures of DNA and RNA of bacteria so that the bacteria can be inactivated (Cutler and Zimmerman, 2011). Furthermore, a styrene molecule also consists of a double-bond and a conjugated ring. Thus, styrene was selected as a reductant for this experiment. Besides, the individual effect of  $O_3$  and UVC-LED on the reduction of styrene was also considered for a comparison. The experimental procedure is shown in Fig. 1.

Five integrated cases of UVC-LED and  $O_3$  were conducted. Experimental conditions are presented in Table 1. A 1 L Tedlar bag (SKC Inc., USA) was used as a reaction chamber. Styrene and  $O_3$  were taken from their standard source and introduced into the bag by 100 mL glass syringes (Fig. 1) in order to produce 500 mL sam-

ple gas. First styrene was injected into the bag by a syringe, and after a while  $O_3$  was injected. Since target gases at ppmv level were injected into the bag by syringes which caused a turbulent air inside the bag, these gases could mix well in the bag. After each experiment, a new bag was used to prevent the contamination. Particularly, when styrene was exposed by only UVC-LED, zero air was injected into the bag instead of  $O_3$ . After exposing styrene to oxidizing agents, styrene in the bag was introduced into an adsorption trap, then it was analyzed by an GC/MS/TD system. Method detection limit for the chemical observed from the repetition of the first point of the standard series was found to be 3.36 ng.

Each condition was repeated three times. Relative standard deviations of the repetition were less than 7%. Average removal efficiencies of styrene at each condition were obtained and compared. ANOVA and t-test were conducted using Statgraphics Centurion XV soft-

**Table 1.** Experimental conditions for styrene with various integrated cases of UVC and O<sub>3</sub>.

Code	Integrated case	Styrene (ppmv)	Ozone (ppmv)	UVC* (mW/cm <sup>2</sup> )	Exposure time (min)
UV	Exposed by only UVC-LED	194	0	0.77	10
O <sub>3</sub>	Exposed by only O <sub>3</sub>	194	100, 200	0	10
UV& O <sub>3</sub>	Exposed simultaneously by O <sub>3</sub> and UVC-LED	194	100, 200	0.77	10
O <sub>3</sub> > UV	Exposed by O <sub>3</sub> , then by UVC-LED	194	100, 200	0.77	5 : 5
UV> O <sub>3</sub>	Exposed by UVC-LED, then by O <sub>3</sub>	194	100, 200	0.77	5 : 5

Note: \* UVC dose inside the Tedlar bag.

**Table 2.** Experimental conditions for bioaerosol with various integrated cases of UVC and O<sub>3</sub>.

Code	Integrated case	Ozone (ppmv)	UVC* (mW/cm <sup>2</sup> )	Exposure time (min)
UV	Exposed by only UVC-LED	0	0.77	1, 30
O <sub>3</sub>	Exposed by only O <sub>3</sub>	100, 200	0.77	1, 30
O <sub>3</sub> > UV	Exposed by O <sub>3</sub> , then by UVC-LED	100, 200	0.77	0.5 : 0.5, 15 : 15

Note: \*UV dose inside the Tedlar bag.

ware (15.2.05; Statpoint Technologies Inc., Warrenton, VA, USA).

### 2.3.2 The Effects of the Integrated O<sub>3</sub>/UVC-LED Treatment on the Inactivation of Bacteria in Liquid Droplets

From experimental results with styrene, the effect of the integrated O<sub>3</sub>/UVC-LED treatment on bacteria in droplets in a batch stage were carried out with respect to UV, O<sub>3</sub>, and O<sub>3</sub>> UV conditions. Conditions which were UV&O<sub>3</sub> and UV> O<sub>3</sub> were not depicted here due to their low efficiencies. Droplets of bacteria were produced by depositing 10 small droplets (i.e., 100 µL per droplet) of solution containing *Salmonella typhimurium* bacteria on a sterilized glass (7 cm × 2 cm). The sample was dried at room temperature (25°C ± 1°C) for 60 minutes to reduce the amount of water. After that, each sample was inserted into a 1 L Tedlar bag containing 500 mL of zero air or O<sub>3</sub> at 100 ppmv and 200 ppmv. The experimental procedure is presented in Fig. 1. Experimental conditions are shown in Table 2.

After being exposed by oxidizing agents, the plates were washed with distilled water. *Salmonella typhimurium* in each sample was inoculated, cultured, and counted. The reduction ratio of bacteria was evaluated based on equation (2).

$$\text{Bacteria reduction ratio} = \frac{(N_{in} - N_{at})}{N_{in}} \times 100\% \quad (2)$$

where  $N_{in}$  is the initial number of bacteria before treatment (CFU/mL),  $N_{at}$  is the number of bacteria after treatment (CFU/mL). In this study, the initial number of bacteria before treatment was the number of bacteria in 10 droplets after drying 60 minutes. The number of bacteria in the original solution was not used because the number of bacteria would obviously be reduced after drying 60 minutes. In other words, the initial number of bacteria before treatment was the value of the blank sample.

Each experiment was repeated two times. Relative percent differences of results between two experimental events were less than 13%. ANOVA and t-test were conducted using a Statgraphics Centurion XV software (15.2.05; Statpoint Technologies Inc., Warrenton, VA, USA).

## 3. RESULTS AND DISCUSSION

### 3.1 Investigation on Interaction between O<sub>3</sub> and UVC-LED

The reduction rates of O<sub>3</sub> caused by UVC-LED were investigated with respect to various exposure times. Experimental results are shown in Fig. 2.

As shown in Fig. 2, reduction rates of O<sub>3</sub> caused by 0.77 mW/cm<sup>2</sup> of UVC-LED were proportional to the exposure times, inversely proportional to O<sub>3</sub> concentrations. Reduction rates of 100 ppmv and 200 ppmv O<sub>3</sub> from 1 minute to 15 minutes were dramatically increased from 15 and 55% to 70 and 80%, respectively. In contrast, those were slightly increased from 70 and 80% to 84 and 89% from 15 to 30 minutes, respectively. It suggested that UVC-LED can be employed to remove O<sub>3</sub>. However, the effect of UVC-LED could be declined over time. Hence, to remove well O<sub>3</sub>, the intensity of UVC-LED should be increased rather than the contact time.

Interactions between O<sub>3</sub> and UVC-LED were investigated using styrene as a representative compound. Experimental results are depicted in Fig. 3.

As shown in Fig. 3, the styrene removal efficiency by only UVC showed the lowest value (i.e., 6.33%). This low degradation was due to a short exposure time (Lim *et al.*, 2008; Kodaira *et al.*, 1973). A t-test between integrated method with respect to 100 ppmv O<sub>3</sub> and 200 ppmv O<sub>3</sub> was conducted with 95% confidence in order to figure out the significant difference. It was found that styrene removal efficiency by integrated methods with respect to 200 ppmv O<sub>3</sub> (i.e., O<sub>3</sub>, UV&O<sub>3</sub>, O<sub>3</sub>>UV and UV>O<sub>3</sub>) revealed a significant difference to those with respect to 100 ppmv O<sub>3</sub> because of P-values < 0.05. At 200 ppmv O<sub>3</sub>, the removal efficiency of styrene was approximately 100%, which indicated that styrene was almost oxidized fully. An ANOVA test was conducted among integrated methods (i.e., O<sub>3</sub>, UV&O<sub>3</sub>, O<sub>3</sub>>UV and UV>O<sub>3</sub>) with respect to 200 ppmv O<sub>3</sub>, which showed that there was no significant difference in styrene removal efficiencies among these methods due to P-value = 0.4006. Therefore, integrated methods with respect to 200 ppmv O<sub>3</sub> should not be used to get the optimal integrated way. In terms of integrated methods associated with 100 ppmv O<sub>3</sub>, the method in which styrene at first was exposed to O<sub>3</sub> for 5 minutes then to UVC-LED for 5 minutes (i.e., O<sub>3</sub>>UV) revealed the highest removal efficiency, which was followed by O<sub>3</sub>, UV&O<sub>3</sub>, and UV>O<sub>3</sub> (P-value = 0.0221). When styrene was exposed to O<sub>3</sub> and UVC-LED at the same time, the removal efficiency was lower than that being exposed by only O<sub>3</sub> because UVC could destroy O<sub>3</sub> (Summerfelt, 2003; de Gruijil and van der Leun, 2000), and O<sub>3</sub> could absorb UVC (Keller-Rudek *et al.*, 2013). These effects caused the reduction of UVC-LED effectiveness.

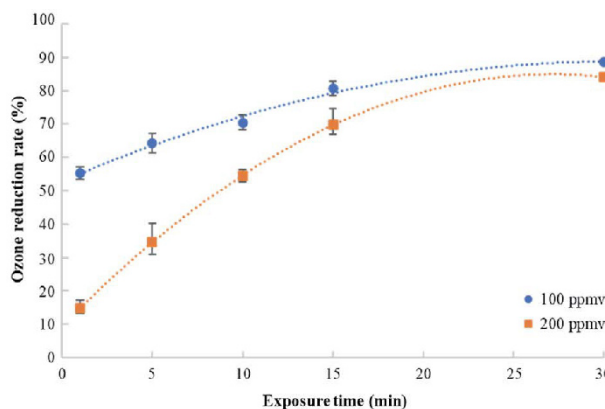


Fig. 2. Reduction rates of O<sub>3</sub> associated with various exposure times to UVC-LED (Error bars show minimum and maximum values).

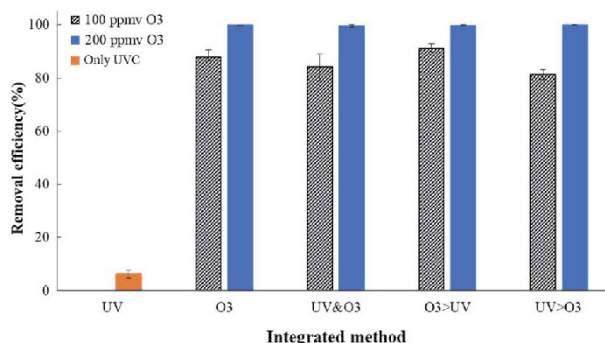
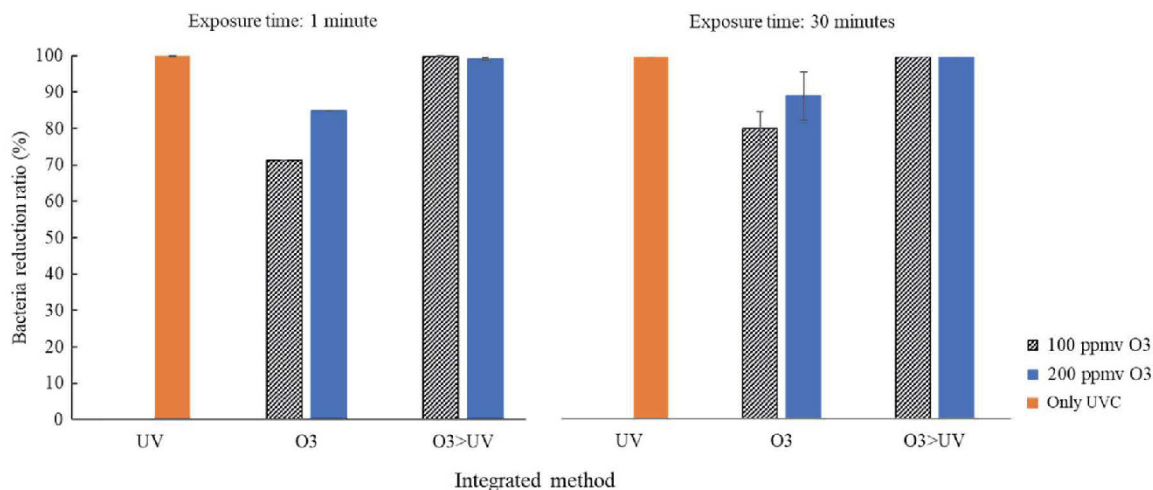


Fig. 3. Removal efficiencies of styrene with respect to various integrated O<sub>3</sub> and UVC-LED treatments and two O<sub>3</sub> concentrations. (Error bars show minimum and maximum values).

Consequently, O<sub>3</sub> and UVC-LED should not be employed at the same time. Between O<sub>3</sub>>UV and UV>O<sub>3</sub>, experimental results suggested that O<sub>3</sub>>UV is more suitable for inactivating bacteria or viruses in bioaerosols because this method will be applied for the indoor air while O<sub>3</sub> is a toxic compound for human beings, especially over 100 ppbv (Pohanish, 2012). As a suggestion method, O<sub>3</sub> can firstly disinfect a part of bioaerosols. After that, UVC-LED can be used to remove the rest of bioaerosols as well as to decompose O<sub>3</sub>. However, the investment cost and benefit should be considered when both of O<sub>3</sub> and UVC-LED are employed. For example, integrated O<sub>3</sub> and UVC-LED can be used to remove volatile organic compounds and bioaerosols together. The reduction rates of O<sub>3</sub> depended on UVC dose and contact time (Fig. 2). More studies with higher



**Fig. 4.** Bacteria reduction ratios with respect to various integrated methods and exposure times (Error bars show minimum and maximum values).

UVC doses should be conducted to figure out the optimal condition which could remove O<sub>3</sub> to a negligible level in future works.

### 3.2 The Effects of the Integrated O<sub>3</sub>/UVC-LED Treatment on the Inactivation of Bacteria in Liquid Droplets

Droplets of *Salmonella typhimurium* bacteria were exposed to O<sub>3</sub> and UVC-LED to determine the effect of individual and optimal integration approaches on bacteria. A condition of O<sub>3</sub> > UV was taken into account in this experiment based on the results of the previous experiment.

Bacteria reduction ratios with respect to various oxidation conditions and exposure times are presented in Fig. 4.

Contrary to the experiment of styrene, UVC-LEDs showed the best reduction ratio of bacteria (>99.99%) with respect to both exposure times (Fig. 4). Although the exposure time was only 1 min, this result was better than that of a previous study which needed more than 1 hour exposure (Nunayon *et al.*, 2020). However, the distance between UVC-LEDs and bacteria in this current study was only 3 cm while UVC irradiation diameter of the previous study was 2.01 m (Nunayon *et al.*, 2020). Hence, it suggested that to increase the reduction efficiency and reduce the irradiation time, many UVC-LEDs should be used and arranged with a closer distance. Bacteria reduction efficiencies of O<sub>3</sub> showed lower values than those of UVC-LEDs. These low effi-

ciencies might be due to different phase like O<sub>3</sub> gas and liquid droplets. Since exposure times in the current study were shorter than those of previous studies, O<sub>3</sub> had not enough time to inactivate all bacteria. Masotti *et al.* (2019) reported that it took three hours to disinfect air-borne bacteria by 5 ppmv O<sub>3</sub> (Masotti *et al.*, 2019). When 150 ppmv O<sub>3</sub> was used to disinfect bacteria including *E. coli* and *B. subtilis* in bioaerosols, the reduction ratio of total bacteria was approximately 93.7% after 2 h with an air change rate of 3.89 L/h (Huang *et al.*, 2012). When using 80 ppmv O<sub>3</sub> to treat bioaerosol containing viruses, it took three hours to reach 99.9% (Steinmann *et al.*, 2021). In addition, droplets used in the current study also comprised the nutrient broth which could react with O<sub>3</sub>, so that it might reduce the effect of O<sub>3</sub> on bacteria. The experiment was conducted in a batch condition and the contacting surface between gas phase and liquid phase of 10 droplets was also limited and constant due to small droplet deposited on a glass plate. Therefore, the dissolving rate of O<sub>3</sub> to the droplet was limited although O<sub>3</sub> could be dissolved well in water. This might be a reason why the bacteria reduction rate was dramatically increased at the beginning (i.e., within 1 minutes) but slightly increased approximately by 10% for longer exposure times. This pattern was also found in a previous study. When 150 ppmv O<sub>3</sub> was applied to a 36.5 m<sup>3</sup> room, it took about 70 minutes to reduce 87.5% total bacteria and spent 50 minutes more to reach only 93.7% of reduction rate (Huang *et al.*, 2012). In terms

of integrated O<sub>3</sub>/UVC-LED treatment methods, there was no significant difference in bacteria reduction ratios (>99%) between 100 and 200 ppmv O<sub>3</sub> for 1 min exposure (P-value = 0.4197). Although O<sub>3</sub> was combined with UVC-LED, bacteria reduction ratios were lower than those of only UVC-LED. This effect could be found very clear when the exposure time was 1 minute. While the reduction ratio with respect to only UVC-LED was >99.9% (i.e., N<sub>at</sub> = 65 CFU/mL) after 1 minute exposure, the reduction ratio of O<sub>3</sub> > UV was 99.8% (i.e., N<sub>at</sub> = 8,800 CFU/mL) at 100 ppmv O<sub>3</sub>, but that was only 99.1% (i.e., N<sub>at</sub> = 55,000 CFU/mL) at 200 ppmv O<sub>3</sub>. This pattern again confirmed the effect of O<sub>3</sub> on the performance of UVC-LED. The 30-minute exposure test showed a reduction rate of over 99.99% for both ozone concentrations. This result was mainly due to UVC-LED because only O<sub>3</sub> showed low reduction ratios (<90%).

In general, UVC-LED revealed a high reduction ratio of bacteria in droplets when they were located close to the droplets. When O<sub>3</sub> was combined with UVC-LED, the exposure of O<sub>3</sub> and UVC-LED should be separated because they could interact each other, so that their performance would be reduced. Since the current study was carried out with 1 minute and 30 minutes of exposure times, they were not enough for the complete inactivation of bacteria in droplets; therefore it was hard to figure out the best exposure time of O<sub>3</sub> for bacteria before introducing it to UVC-LED. Various exposure times should be concerned in future works. In addition, UVC dose was fixed and had a relatively high value in this study (i.e., identical UVC intensity and distance), so that O<sub>3</sub> could not show its full inactivation potential. More studies with different UVC doses by changing the number of LEDs or the distance of LEDs and bacteria should be implemented. *Salmonella typhimurium* bacterium was used as a representative germ in this study.

#### 4. CONCLUSIONS

O<sub>3</sub> and UVC were integrated in various ways to investigate their effects on the inactivation of bacteria in liquid droplets. UVC-LED at 275 nm were applied while the O<sub>3</sub> concentrations varied as 100 and 200 ppmv. Using only O<sub>3</sub>, using only UVC, exposing O<sub>3</sub> and UVC at the same time, exposing O<sub>3</sub> first and then UVC, and exposing UVC first and then O<sub>3</sub> were considered as various integrated methods. For the interaction between O<sub>3</sub> and

UVC-LED, styrene 194 ppmv was used as an oxidizing compound. For the effect of O<sub>3</sub>/UVC-LED on bacteria, *Salmonella typhimurium* solution was employed. It was found that the performance of UVC-LED and O<sub>3</sub> was reduced only when they were applied at the same time. UVC-LED could reduce approximately 90% of 100 ppmv O<sub>3</sub> after 30 minutes exposure time in this study. For styrene, O<sub>3</sub> revealed a better reduction ratio than that of UVC-LED. In contrast, UVC-LED could reduce over 99.99% of bacteria while O<sub>3</sub> did less than 90%. This study suggested that individual UVC-LED could disinfect bacteria in droplets rather than individual O<sub>3</sub>. On the other hand, if O<sub>3</sub> and UVC-LED are applied to improve indoor air quality such as treating volatile organic compounds and bioaerosol, the integrated method in which bioaerosol was exposed to O<sub>3</sub> first, then to UVC-LED should be practically applied to minimize the interaction between them and improve the removal efficiencies. However, this study still has some limitations with respect to exposure times, UVC doses, and the types of germs. Thus, these issues should be more studied in the future works.

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#### REFERENCES

- Acuti Martellucci, C., Flacco, M.E., Cappadona, R., Bravi, F., Mantovani, L., Manzoli, L. (2020) SARS-CoV-2 pandemic: An overview. *Advances in Biological Regulation*, 77, 100-736. <https://doi.org/10.1016/j.jbbior.2020.100736>
- Batakliev, T., Georgiev, V., Anachkov, M., Rakovsky, S., Rakovsky, S. (2014) Ozone decomposition. *Interdisciplinary Toxicology*, 7, 47-59. <https://doi.org/10.2478/intox-2014-0008>
- Beck, S.E., Ryu, H., Boczek, L.A., Cashdollar, J.L., Jeanis, K.M., Rosenblum, J.S., Lawal, O.R., Linden, K.G. (2017) Evaluating UV-C LED disinfection performance and investigating potential dual-wavelength synergy. *Water Research*, 109, 207-216. <https://doi.org/10.1016/j.watres.2016.11.024>
- Buising, K., Schofield, R., Irving, L., Keywood, M., Stevens, A., Keogh, N., Skidmore, G., Wadlow, I., Kevin, K., Rismanchi, B., Wheeler, A., Humphries, R., Kainer, M., McGain, F., Monty, J., Marshall, C. (2021) Use of portable air cleaners to reduce aerosol transmission on a hospital COVID-19 ward. *medRxiv*. <https://doi.org/10.1101/2021.03.29.21254590>



- Cutler, T.D., Zimmerman, J.J. (2011) Ultraviolet irradiation and the mechanisms underlying its inactivation of infectious agents. *Animal Health Research Reviews*, 12, 15–23. <https://doi.org/10.1017/S1466252311000016>
- da Costa Filho, B.M., Vilar, V.J.P. (2020) Strategies for the intensification of photocatalytic oxidation processes towards air streams decontamination: A review. *Chemical Engineering Journal*, 391, 123531. <https://doi.org/10.1016/j.cej.2019.123531>
- de Grujil, F.R., van der Leun, J.C. (2000) Environment and health: 3. Ozone depletion and ultraviolet radiation. *Canada Medical Association Journal*, 163, 851–855.
- Franke, G., Knobling, B., Brill, F.H., Becker, B., Klupp, E.M., Belmar Campos, C., Pfefferle, S., Lütgehetmann, M., Knobloch, J.K. (2021) An automated room disinfection system using ozone is highly active against surrogates for SARS-CoV-2. *Journal of Hospital Infection*, 112, 108–113. <https://doi.org/10.1016/j.jhin.2021.04.007>
- Huang, H.-L., Lee, M.-G., Tai, J.-H. (2012) Controlling Indoor Bioaerosols Using a Hybrid System of Ozone and Catalysts. *Aerosol and Air Quality Research*, 12, 73–82. <https://doi.org/10.4209/aaqr.2011.07.0098>
- Keller-Rudek, H., Moortgat, G.K., Sander, R., Sørensen, R. (2013) The MPI-Mainz UV/VIS Spectral Atlas of Gaseous Molecules of Atmospheric Interest. *Earth System Science Data*, 5, 365–373. <https://doi.org/10.5194/essd-5-365-2013>
- Kodaira, T., Hayashi, K., Ohnishi, T. (1973) Photopolymerization of Styrene in the Presence of Oxygen. Role of the Charge-Transfer Complex. *Polymer Journal*, 4, 1–9. <https://doi.org/10.1295/polymj.4.1>
- Kruza, M., McFiggans, G., Waring, M.S., Wells, J.R., Carslaw, N. (2020) Indoor secondary organic aerosols: Towards an improved representation of their formation and composition in models. *Atmospheric Environment*, 240, 117784. <https://doi.org/10.1016/j.atmosenv.2020.117784>
- Lee, J.-Y., Dinh, T.-V., Kim, D.-J., Choi, I.-Y., Ahn, J.-W., Park, S.-Y., Jung, Y.-J., Kim, J.-C. (2019a) Comparison of Water Pretreatment Devices for the Measurement of Polar Odorous Compounds. *Applied Sciences*, 9, 4045. <https://doi.org/10.3390/app9194045>
- Lee, J.-Y., Dinh, T.-V., Kim, D.-J., Choi, I.-Y., Ahn, J.-W., Park, S.-Y., Jung, Y.-J., Kim, J.-C. (2019b) Effect of Conventional Water Pretreatment Devices on Polar Compound Analysis. *Asian Journal of Atmospheric Environment*, 13, 249–258. <https://doi.org/10.5572/ajae.2019.13.4.249>
- Lim, M., Rudolph, V., Anpo, M., (Max) Lu, G.Q. (2008) Fluorinated-bed photocatalytic degradation of airborne styrene. *Catalysis Today*, 131, 548–552. <https://doi.org/10.1016/j.cattod.2007.10.092>
- Masotti, F., Vallone, L., Ranzini, S., Silvetti, T., Morandi, S., Brasca, M. (2019) Effectiveness of air disinfection by ozonation or hydrogen peroxide aerosolization in dairy environments. *Food Control*, 97, 32–38. <https://doi.org/10.1016/j.foodcont.2018.10.022>
- Mohamed, Z., Barbara, R. (2021) Inactivation of microbes by ozone in the food industry: A review. *African Journal of Food Science*, 15, 113–120. <https://doi.org/10.5897/AJFS>
- 2020.2074
- Morawska, L., Tang, J.W., Bahnfleth, W., Bluysen, P.M., Boerstra, A., Buonanno, G., Cao, J., Dancer, S., Floto, A., Franchimon, F., Haworth, C., Hogeling, J., Isaxon, C., Jimenez, J.L., Kurnitski, J., Li, Y., Loomans, M., Marks, G., Marr, L.C., Mazzeo, L., Melikov, A.K., Miller, S., Milton, D.K., Nazaroff, W., Nielsen, P.V., Noakes, C., Peccia, J., Querol, X., Sekhar, C., Seppänen, O., Tanabe, S., Tellier, R., Tham, K.W., Wargo, P., Wierzbicka, A., Yao, M. (2020) How can airborne transmission of COVID-19 indoors be minimised? *Environment International*, 142, 105832. <https://doi.org/10.1016/j.envint.2020.105832>
- Nunayon, S.S., Zhang, H.H., Lai, A.C.K. (2020) A novel upper-room UVC-LED irradiation system for disinfection of indoor bioaerosols under different operating and airflow conditions. *Journal of Hazardous Materials*, 396, 122715. <https://doi.org/10.1016/j.jhazmat.2020.122715>
- Ploydaeng, M., Rajatanavin, N., Rattanakaemakorn, P. (2021) UV-C light: A powerful technique for inactivating microorganisms and the related side effects to the skin. *Photodermatology Photoimmunology & Photomedicine*, 37, 12–19. <https://doi.org/10.1111/phpp.12605>
- Pohanish, R.P. (2012) *Sittig's Handbook of toxic and hazardous chemicals and carcinogens*, sixth. ed. Elsevier Inc., Oxford.
- Qian, H., Miao, T., Liu, L., Zheng, X., Luo, D., Li, Y. (2021) Indoor transmission of SARS-CoV-2. *Indoor Air*, 31, 639–645. <https://doi.org/10.1111/ina.12766>
- Ren, Y., Li, L., Jia, Y. (2020) New Method to Reduce COVID-19 Transmission - The Need for Medical Air Disinfection is Now. *Journal of Medical Systems*, 44, 119. <https://doi.org/10.1007/s10916-020-01585-8>
- Sallustio, F., Cardinale, G., Voccola, S., Picerno, A., Porcaro, P., Gesualdo, L. (2021) Ozone eliminates Novel Coronavirus Sars-CoV-2 in mucosal samples. *New Microbes New Infections*, 100927. <https://doi.org/10.1016/j.nmni.2021.100927>
- Shi, Q., Chen, Z., Liu, H., Lu, Y., Li, K., Shi, Y., Mao, Y., Hu, H.-Y. (2021) Efficient synergistic disinfection by ozone, ultraviolet irradiation and chlorine in secondary effluents. *Science of Total Environment*, 758, 143641. <https://doi.org/10.1016/j.scitotenv.2020.143641>
- Steinmann, J., Burkard, T., Becker, B., Paulmann, D., Todt, D., Bischoff, B., Steinmann, E., Brill, F.H.H. (2021) Virucidal efficacy of an ozone-generating system for automated room disinfection. *Journal of Hospital Infection*, 116, 16–20. <https://doi.org/10.1016/j.jhin.2021.06.004>
- Stetzenbach, L.D. (2009) Airborne Infectious Microorganisms, in: *Encyclopedia of Microbiology*. Elsevier, pp. 175–182. <https://doi.org/10.1016/B978-012373944-5.00177-2>
- Summerfelt, S.T. (2003) Ozonation and UV irradiation – an introduction and examples of current applications. *Aquacultural Engineering*, 28, 21–36. [https://doi.org/10.1016/S0144-8609\(02\)00069-9](https://doi.org/10.1016/S0144-8609(02)00069-9)
- US EPA (2021) Indoor Air and Coronavirus (COVID-19). Available online: <https://www.epa.gov/coronavirus/indoor-air-and-coronavirus-covid-19/> (accessed on 10 August 2021).

- Wang, J., Zhang, Y., Yu, Y., Wu, Z., Wang, H. (2021) Combination of ozone and ultrasonic-assisted aerosolization sanitizer as a sanitizing process to disinfect fresh-cut lettuce. *Ultrasonics Sonochemistry*, 76, 105622. <https://doi.org/10.1016/j.ultsonch.2021.105622>
- Wengraitis, S., McCubbin, P., Wade, M.M., Biggs, T.D., Hall, S., Williams, L.I., Zulich, A.W. (2013) Pulsed UV-C Disinfection of *Escherichia coli* With Light-Emitting Diodes, Emitted at Various Repetition Rates and Duty Cycles. *Photochemical and Photobiology*, 89, 127–131. <https://doi.org/10.1111/j.1751-1097.2012.01203.x>
- WHO (2021a) Coronavirus disease (COVID-19) pandemic. Available online: <https://www.who.int/emergencies/diseases/novel-coronavirus-2019> (accessed on 10 August 2021).
- WHO (2021b) Coronavirus disease (COVID-19): How is it transmitted? Available online: <https://www.who.int/news-room/q-a-detail/coronavirus-disease-covid-19-how-is-it-transmitted/> (accessed on 10 August 2021).
- WHO (2021c) Coronavirus disease (COVID-19): Ventilation and air conditioning. Available online: <https://www.who.int/news-room/q-a-detail/coronavirus-disease-covid-19-ventilation-and-air-conditioning> (accessed on 10 August 2021).
- Yoo, S.T., Lee, J.Y., Rodiansyah, A., Yune, T.Y., Park, K.C. (2021) Far UVC light for *E. coli* disinfection generated by carbon nanotube cold cathode and sapphire anode. *Current Applied Physics*, 28, 93–97. <https://doi.org/10.1016/j.cap.2021.05.007>